



## Diagnosis of *Hordeum vulgare* Genomic Profile: Review

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### Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/APRJ/2021/v8i330178

Editor(s):

(1) Dr. Vinayaka K.S, Kumadvathi First Grade College, India.

Reviewers:

(1) Radoslaw Janusz Walkowiak, International Equisetological Association, Poland.

(2) Cardei Petru, The National Institute of Research, Romania.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/75940>

**Received 14 August 2021**

**Accepted 29 October 2021**

**Published 03 November 2021**

**Review Article**

### ABSTRACT

This review represent plants genetic diversity (PDG) generally in crop plant and especially in Barley (*Hordeum vulgare*), can be studied (PDG) and stored as a (PGR) plant genetic resources as gene bank , DNA library for saved genetic material at long time and crops improvement can be utilized in breeding programs strategies in future. In this study observed the significance of plant genetic diversity (PGD) and (PGR) especially on agriculturally important crops , analysis of plant genomic using molecular markers. Barley is a well important studies crops using as a model for study genetic plant, cultivated barley *Hordeum vulgare* easily hybridization by genetic fingerprinting with wild barley *Hordeum spontaneum*. The molecular markers showed their relation with locus of geographic factors and imposed stresses. Here, discussed barley genomic through relationship between genotype and phenotype traits using molecular markers useful for genetic physiological maps construction.

**Keywords:** Crop plant; barley; molecular markers; genetic diversity.

### 1. INTRODUCTION

The genetic variability has been used naturally through agriculture crops to provided food

requirement, here it is being focused to accumulate food for individuals growing. Barley crop production in the 21<sup>st</sup> century about 2 billion tons (according to FAO in 2006;

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<http://www.fao.org>) that take four degree among cereals and used as a healthy food, feed grain and malting drink production. The study of crops genetic origin can be through isolate agronomical important genes from the genetic variability present in the gene of barley. It has been observed during the last two decades development genome based technologies that easily to diagnosis genome of barley their include molecular markers [1,2]. Barley is the most economic cereal crop that is tolerant to climate variations (Fig. 1), (Fig. 2).

It is cultivated in regions variety representing a wide range of temperatures, and tolerates drought, low temperatures and salinity [3,4]. It is used in human nutrition and beer making. In developing countries in marginal environments such as highlands and the edges of deserts. It is considered the oldest cereal crop cultivated and is cultivated to obtain its grain, which is the most important forage resource, in addition to being

used as green fodder to feed livestock, and barley straw is used as a filler in Animal diet was used in the past as human food in some European societies. The largest use of barley was in Morocco (61%), Ethiopia (79%), China (62%), and India (73%) [5]. The barley plantation occupies an area of 8.7 million hectares and the production of 9 million hectares in the Arab country, and the most important Arab countries in terms of area and production: Morocco (3.4 million hectares), Syria (600 thousand , Algeria (1 million hectares), and Iraq (2.5 million hectares). Drought is the main determinant factor in barley production and affects production just like other environmental stresses, as the lack of water available to the plant is caused. The decline in grain yield and productivity in the various regions of barley cultivation in the world Because of the increased risk of drought, improving barley to withstand drought becomes important and vital [6,7].



Fig. 1. Barley (*Hordeum vulgare*) crop



Fig. 2. Barley samples of 4 spike types: a, 6- row. b, 4- row. c & d, 2- row

Plant roots provide the shoots with the water needed for life, and the ideal root system can improve the plant's phenotypic properties, which in turn improve grain yield, increase nutrient absorption and tolerance drought [8,9]. There are several factors that contribute to barley's tolerance to drought, including root depth, number of primary roots, size and diameter of woody vessels [10]. The primary roots exhibit high penetration potential when the number and diameter of their woody vessels increase, and accordingly more than 1000 genotypes that are drought tolerant and have a high heritability have been selected [11]. It can be said that barley is an ideal model for cereal crops due to the widely available genetic information about it [12]. Improving barley's tolerance to environmental stress depends to a large extent on exploiting the genetic variations available in cultivated varieties (*Hordeum vulgare*) and wild barley (*Hordeum spontaneum*) [13]. We also, knowledge the genetic diversity of genetic resources and the genetic relationships between them constitute basic information for plant breeders for their usefulness in designing genetic breeding and improvement programs and in improving the efficiency of the management of genetic assets and preserving them from genetic drift [14]. On the other hand, the growing interest in understanding linkage disequilibrium LD and developing methods of calculating it provides the possibility of using it in detecting the association between genetic indicators and phenotypic traits, LD-based participatory analyzes were used for the first time to locate the QTL gene responsible for some human diseases [15,16]. The linkage disequilibrium LD It can be more powerful and accurate than traditional linkage analysis in detecting mutations with a weak effect associated with some diseases [17]. The traditional linkage analysis uses two-parent crosses and thus does not allow the study of the limited differences between parents used in developing any isolation group, That is, the study is limited to the molecular markers and genetic sites that show a difference between parents [18]. While LD method avoided all the drawbacks of traditional correlation analysis, by using a variety of non-genetically related inputs such as varieties, strains and hybrids, this method provides a broad base of genetic diversity and therefore a very large number of traits are different in the input set used, which allows studying all these traits together in the group. Available large number of different indicators between the inputs. In addition to all this, the availability of information and data on

the phenotypic characteristics of the inputs, the improved strains, and the cultivated varieties allows studying the link between these traits and molecular markers, thus saving much effort and time needed to conduct new experiments and develop suitable isolation groups [19]. In this paper we will study progress of functional and structural process of barley gene due to the importance this crop and the knowledge of its genetic diversity, depending on some formal and productive characteristics and linking them with molecular markers. To with development of new biotechnology techniques for long decades than classical breeding programs for expanding the genetic base selecting the differentiated genetic varieties and introducing it into the crossbreeding programs.

### 1.1 Functional Genomics of Barley

Currently, it has become easy to study the functions of the barley gene using genetic tools and to clarify the importance of genetic engineering and its role in manipulating genetic content for the purpose of improving the genetic and functional characteristics of plant cells and tissues of barley crop in future.

### 1.2 Reverse Genes

been developed in barley [20] such as: . TILLING(targeting induced local lesions in genomes) [21] then and IMS (insertion mutagenesis system) [22]. Thus, the Scottish Crop Research Institute generated a large M2 TILLING population in the barley cultivar "Optic" with leaf material and seeds from 20,000 20 000 plants freeze dried and archived [21]. To observed analysis of gene functions , the IMS method used in barley through past decade by: generate loss function mutations including insertion transposable material converted to importance gene [23] and create dominant mutations gain function using gene insertion randomly either sequence enhancer or sequence promoter [24,25]. insertion lines creating in GMO plant specificity in barley observed single copy Ds launch lunch pads insertion lines in barley genome can be used as a targeted gene tagging in future [26]. Dominant overexpression of phenotypes help to study gene function in barley genome , where loss of function mutations occur that resulted to no phenotypes because gene frequency.

#### 1.2.1 Transgenic barley and its applications

The idea of create genetic transformation technique in barley that necessary for functional

characterization of specific genes used in genetic studies. genetic transformation method was efficiency and stable that developed in spring barley its applicator based on immature embryos [27] and then transformation developed in winter barley for improved this technique through with *Agrobacterium* infection in cultures of androgenic pollen, resulted homozygous haploid plants direct with high quantities during chromosome doubling [28]. recently Protein engineering method used to enhance the feed quality for animals through transgenic to generated thermo stable of grains barley (1, 3; 1, 4 $\beta$ -glucanases) [29,30] Then recently in laboratory can be diagnosis a new protein called Jekyll, that expressed through nuclear projection in barley grain tissue [31]. Its decreased regulation autolysis of nurse tissue, it is produced proliferation of endosperm nuclei with few starch that assembled in endosperm [32].

### **1.3 Genetic Diversity in Crop Plants**

The diagnosis of genetic diversity between plant individuals is worked using techniques such as: morphological traits, biochemical analyses, and molecular markers. First type Morphological tool can be identify by seen features as color of leaf and flower, shape of seed, growth of plant and pigmentation. This technique not costly but requires large agriculture area. These traits was environmental various that important also in genotypic polymorphism. These markers can be distinguished within plant populations between adult plant from sick genetic contamination plant. Second type biochemical analysis that included allelic enzyme as isozymes can be diagnosed using electrophoresis method with specific staining, to performed this analysis required small material of plant sample for detection its resulted isozyme codominant at level of function genetic have simple inheritance. This types of technique considered limitation because of limited numbers of isozymes that complex shape of its structure with specific problems, Therefore this makes it difficult to analyze genetic diversity [33].

Third type molecular genetic markers that included nucleic acid techniques that widely range can be used DNA material to assessment molecular diversity between individuals by analysis of genome and detection cases of deletion, insertion, translocation, and duplication in chromosomes. These markers located near of genes therefore do not effect on phenotypes traits but only controlling on this traits, and this

markers inherited both dominant and codominant patterns. Molecular markers represent as genomic locus, identify by employed specific primers or probe can be distinguished chromosomal traits as well as flanking region at the 3' and 5' extremity. Molecular markers can or cannot related with phenotypic expression of a genomic features. Advantages of these markers are stable and detectable in all organisms regardless of growth, differentiation, development [34].

Here in this paper we are not describing much about the pre genomic tools but study genomic development and its assistance genetic diversity diagnosis in crops.

### **1.4 Development of Molecular Markers**

DNA markers are broadly used from past to now, they create from different types of mutation occurs in DNA such as: alternative mutation (point mutation), R rearrange mutation (deletion or insertion), or error in tandem repeat of DNA replication [35]. These markers founded at noncoding region in genome therefore considered typically and neutral compared to other markers, DNA markers have been unlimited in number and not effected by environment and development growth of plant populations [36]. DNA markers have numerous fields in plant breeding such as: assessment of genetic diversity level, parental selection, variety identity, identification of cultivar purity, heterosis study and detection of genome [37]. Molecular markers divided in to three types depend on their method diagnosis: (i) hybridization based, (ii) PCR polymerase chain reaction based, (iii) DNA sequencing based.

#### **1.4.1 Restriction fragments length polymorphism (RFLP)**

Restriction fragments length polymorphism technique discovered since 1980 in genetic researches of human [38] and Then were used in plants studies [39]. RFLP marker depend on the differentiation in length of DNA fragments resulted by digestion of DNA gene and hybridization with specific marker in two or more between individuals in population is compared. This technique have been used widely to study genomes between different crop plants wheat, barley, maize, rice oat, and rye [40]. The features of RFLP including diagnosis of unlimited number of locus, codominant, reliable result can be carrying across populations. In otherwise this

markers high cost, required large DNA material, long time work laboratory, and show less polymorphism especial in close relationship lines [41]. RFLP markers were used to develop the first overall molecular marker maps in barley since two decades ago that employed to detection about agronomic quality traits and resistance against several diseases [42,43].

#### **1.4.2 Amplified fragment length polymorphisms (AFLP)**

Marker of amplified fragment length polymorphisms mix between RFLP and PCR analysis. AFLP created by using restriction enzymes to digestion of PCR amplified products through cut the sequence of specific locus in DNA genome. To identify of AFLPs efficiency through observed highly frequency, highly reproductively and fast generation. These advantage making it an Characteristic technique for diagnosed polymorphism and detecting relation between individuals from separated population[44].

#### **1.4.3 Single nucleotide polymorphism (SNPs)**

Single nucleotide polymorphism are variations occurs within in genomic DNA sequence when change in a single nucleotide (A, T, C, or G) in the genome sequence due to variation in genotypes of population. SNPs occur more repeated than any other type of markers are very near to or even within the gene of useful. SNPs are available in the genomes of the most of organisms, including plants, and are widely spread throughout genomes with a variable distribution among species. SNPs technique can be used to rapid identification of crop varieties, study of agronomic or adaptive traits in plant genotypes and construction high density genetic maps based on association genetics studies by two method either DHPLC (denaturing high performance liquid chromatography) or microarray [33]. Other SNP2CAPS programs used to facilitate the conversion of SNP markers into CAPS markers are suggested in [45,46]. Information resulting from various improved mapping projects has been included in development relevance maps [47, 48] which provide perfect genetic information by observing high marker densities. Recently, techniques such as (Illumina Golden Gate Bead Arrays) have also been used at the genome level by scanning SNP-based genotyping platforms [49].

#### **1.4.4 Expressed sequence tag (EST)**

Marker of Expressed sequence tag was alternative source for SSRs marker development as EST based SSR through used study ESTs database [50]. This marker can be fast and no costly via available large number of EST based SSR database compared with development of SSRs genomic sequence [51]. SSRs marker needed long time consuming and expensive of creating genome libraries of high number of clones for founded SSR that contain DNA locus are not require in this approach (Eujayl *et al.*, 2004). EST marker low polymorphism with restriction to species for this type database present compared with SSR genome sequence [52].

#### **1.5 Simple Sequence Repeats (SSR)**

Simple Sequence Repeats techniques also called microsatellite markers widely intensively distribution in genome of individuals , these markers contain from small frequent units. Its presence in eukaryotic genomes and its spread in the coding and non-coding regions in all chromosomes is stated in [53]. There repeated units consist of (1-6) number of nucleotide pairs, that also surrounded by these units clips located in a single area in the genome of same species. Microsatellite e markers can be differentiated between them in their units repeating numbers, type of nucleotide repeat units and its location in genome. SSR markers founded in barley genome [54] that nucleotides pairs frequently every 165Kbp, also repeated units (GA) n and (CA) n of barley genome frequently every 330 and 260Kbp [55]. These markers are diagnosed by high level of polymorphism comparing with other techniques and easily results analysis [52]. This technique is used in many fields such as : genetic map creation , for knowledge of the genetic diversity in plant genotypes of populations , study genetic relationships and detection of variation between species individuals [56]. That considered important information useful for plant breeders in improvement programs and management of genetic origins [57]. The study [58] that documented results to detection genetic polymorphism in Iraqi barley by six primers for genetic screening nine barley varieties, the ratio of polymorphism (80%), total PCR amplification product (11) bands with molecular weight (75-900) bp, the genetic relationship ranged between (0.01098-0.99708). These results grouped in clusters depending on origins and spike type that

useful in biodiversity and breeders rights protection.

### **1.5.1 Inter-simple sequence repeat (ISSR)**

Inter-simple sequence repeat markers using in to detect phylogeny, gene tagging, genetic variation in plant crop such as barley for diagnosis of genotype and genetic mapping creation [59]. ISSR technique included using DNA fragment with primer to amplification places between adjacent, inversely oriented microsatellites, using a single simple sequence repeat (SSR) motifs (di-, tri-,tetra-, or pent nucleotides) containing primers anchored at the 3' or 5' end by two to four arbitrary, often degenerate nucleotides [60]. The advantage of this marker highly polymorphic, fast and efficient in cost, therefore considered low costly compared with AFLP technique, flexibility and no complexity compared with SSR marker, high reproductively compared with RAPD method [61]. The study [62] that reported data's to assessment level phylogenetic between Iraqi barley varieties to assist in breeding and conservation programs of this genotypes by using nine primer that result (41) bands were observed for nine studied items, the percentage of polymorphism (70.5%) with mean of polymorphic band/primer was (4.5) and molecular weight of amplified bands ranged (140-1600) bp, genetic similarity ranged between (0.0854-0.9897) for all barley cultivars. These data results grouped in clusters and sub clusters that provided suitable genetic diversity in hybrid population.

### **1.5.2 Random amplification polymorphism DNA (RAPD)**

Random amplification polymorphism DNA based on PCR application discovered since 1990, considered widely separation useful for study genetic diversity between individuals in population. PCR products of this marker duo to by using one strand of random primer contain about 10 base on both DNA strands with DNA polymerase. The amplification copies can be diagnosed on agarose gel electrophoresis to bands with different molecular weights [63]. The study [64] that registered information to identification genetic diversity between Iraqi barley varieties. This marker using (18) random primers applicator on nine barley genotypes, produced (177) bands with molecular weights (358-4818) bp. the value of polymorphism ranged between (25-100%). Genetic distances ranged from 0.13424 to 0.43789 across barley varieties. Cluster analysis grouped information

that generated from this study for using in programs of barley breeding and improvement.

### **1.6 MAS & QTL**

Marker-assisted selection application considered an important analysis occur when linkage DNA polymorphism that discovered by marker technique with agronomical features for their fast selection in generally breeding programs. MAS can be allowing breeders with the chance to performance of rapid back-crossing, allele enrichment in complex crosses including decrease time and inexpensive for improvement crop plant varieties. MAS detection in barley through resistance disease as a monogenic traits is proposed in [65]. MAS for quantitative traits suffers from two major limitations. (i) Compared to monogenic traits, quantitative traits are characterized by lower heritability's impairing their accurate scoring and entailing a less accurately defined genetic position of the corresponding quantitative trait locus (QTL). As a result, large chromosomal fragment needs to be selected for, resulting in the meiotic transfer of many potentially undesirable gene. Meiotic purification of a QTL into a "mendelian" locus, showing monogenic inheritance, provides a solution to this trouble. In barley crop, this approach has been successfully employed to isolate bot1 gene underlying a major QTL conferring boron tolerance [66], (ii) Many of QTL alleles escape detection, when transferred into different genetic background. The reasons of disappearance of QTLs include epistatic interactions, QTL x environment effects, the allelic states of the parental lines or the small contribution of a single QTL to the overall variance.

Therefore few common QTL where detected when the results of mapping studies that were performed in different crosses were compared [67]. At the end, this marker will allow to treat QTLs as a monogenic traits and thus spur their marker assisted manipulation in breeding program. In combination with a wide range of mapping populations developed for specific agronomic traits, this comprehensive resource of markers now allows the diagnosis of genetic polymorphisms in functionally defined sequences [68,69].

### **1.7 Next Generation Sequencing**

DNA sequencing is detection of the nucleotide bases (A,T,C, and G) in a target fragment of

DNA genome. DNA sequencing technique has played a major role in development of molecular genetic biology [70]. The technology of following generation sequence making a great event in genetic diversity studies for genotypes in population. Helicos are real single sequence molecule technology is a sequencing and genetic analysis method to DNA it shows advantages for traditional and next sequence generation technique. Helicos show the first genetic analysis platform that not needed amplification, through using single molecule of DNA sample preparation process prevent PCR-induced bias and troubles work, simplifies results analysis, and adapts degraded samples. Helicos single-molecule sequencing is often indicate to as third generation sequencing [71,4].

### 1.8 Analysis of Genetic Diversity from Molecular Data

Major methods used to measured data that resulted from molecular application calculations of genetic diversity studies divided in to two major types: (A) analysis of genetic distance or similarity for studied samples (B) parameters calculation of genetic diversity levels between genotypes of populations. The equation for detect of molecular diversity is shown below:

Molecular diversity = Sample × sample pair-relationship (distance and /or similarities)

The study of molecular markers were essential to know genetic relationship among individuals through statistical analysis and quantitative measure of genetic diversity for DNA sequence level and allele frequency depending on resulting data.

To measures of genetic distance (GD) or genetic similarity (GS) are using commonly analysis:

- (i) Nei and Lis [72] coefficient ( $GD_{NL}$ ), (ii) Jaccard's [73] coefficient ( $GD_J$ ), (iii) simple matching coefficient ( $GD_{SM}$ ) [74], and (iv) modified Rogers' distance ( $GD_{MR}$ ). Genetic distance determined by the above measures can be estimated as follows:

$$GD_{NL} = 1 = 2N_{11} / (2N_{11} + N_{10} + N_{01}) ,$$

$$GD_J = 1 = N_{11} / (N_{11} + N_{10} + N_{01}) ,$$

$$GD_{SM} = 1 = (N_{11} + N_{00}) / (N_{11} + N_{10} + N_{01} + N_{00}) ,$$

$$GD_{MR} = (N_{10} + N_{01}) / (2N)$$

Where the symbols represent :  $N$  is the total number of bands/alleles ,  $N_{00}$  is number of bands/alleles absent in both individuals ,  $N_{11}$  is the number of bands/alleles present in both individuals ,  $N_{01}$  is the number of bands/alleles present only in the individual  $j$  ,  $N_{10}$  is the number of bands/alleles present only in the individual  $i$  [75]. There are two main ways of measuring the resulting of genetic relationship matrix called principal coordinate analysis (PCA) and dendrogram or clustering groups, tree pedigree. PCA is used to calculated of genetic distance between samples in the plot that reflect the distances among them with a minimum of distortion by produced 2 or 3 dimensional scatter plot of these samples. Another way represent a dendrogram produced by aggregation of samples with them in clusters groups are to each other than to samples in other clusters.

Genetically same different algorithms method widely used for clustering, include rate (UPGMA) unweight pair group method with arithmetic, neighbour-joining method, and Ward's method [76].

## 2. CONCLUSIONS

Presence of genetic diversity in Barley crop plant is essential for its further improvement by providing options for the breeders to develop new varieties and hybrids. This can be achieved through phenotypic and molecular characterization of PGR by developing tools for measuring the diversity of plant species that are important factors when choosing a technology" . "such as low assay cost, throughput and ease of assay development and automation. Then recently with molecular markers technologies development will generate speed and quality of database, it leading to characterize the larger number of genetic germplasm with limited time and resources. were also produced Next generation sequencing with reduced the cost and time required for sequencing the whole genome, these techniques use for assessing phenotypic, genotypic and molecular diversity parameters that increased the efficiency of genetic germplasm curators, plant breeders to speed up the improvement, and the use of positively correlated with studied traits in creating the genetic link maps. Therefore, we conclude in this paper useful information of molecular genetic tools about Barley that present for widely practical applications to the researchers.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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